REMARKS

Status of the claims

Claims 1-28 and 88-100 were pending in the instant application. With this Response, claims 92 and 93 have been amended; no claims have been canceled; and no claims have been newly added. Upon entry of this paper, therefore, claims 1-28 and 88-100 will be remain pending and under active consideration. Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and reasons that follow.

Objection to the specification

The specification has been objected to for having an embedded hyperlink as part of the specification, which is impermissible according to the M.P.E.P. at § 608.01. With this submission, the hyperlinks in paragraphs 0105 and 0113 on pages 31 and 34, respectively, have been deleted. Hence, Applicants respectfully request that the objection to the specification be withdrawn.

Claim rejections under 35 U.S.C. § 112

Applicants thank the Examiner for acknowledging the withdrawal of the rejections of claims 8 and 28 under 35 U.S.C. § 112, second paragraph, in view of the last-filed Response.

Claims 92 and 93, however, are rejected under the first paragraph of 35 U.S.C. § 112 for allegedly failing to comply with the written description requirement. Specifically, the Examiner alleges that there is only written support for the specific density recited (*i.e.*, 1.0792 or 1.0607) with respect to an iodixanol gradient and not as presented in the amended claims. While Applicants respectfully disagree with the Examiner, Applicants have nonetheless amended claims 92 and 93 to comport with the Examiner's interpretation of the invention. The amendment, however, is not an acquiescence of the propriety of the rejection, and Applicants hereby retain the right to pursue claims to the canceled subject matter in a continuation application.

In view of the amendments to claims 92 and 93, Applicants respectfully submit that the claim rejections have been overcome and respectfully request the withdrawal of same rejections.

Claim rejections under 35 U.S.C. § 102

Claims 1-7, 9-13, 15-21, 26 and 27 stand provisionally rejected under 35 U.S.C. 102(e) as being anticipated by copending application no. 09/764,359 ("the '359 application"). The Examiner alleges that the copending application "teaches methods of isolating liver progenitor cells comprising methods of fractionation by density centrifugation in particular the use of percoll gradients for separation of cell populations from the liver, in particular for the isolation of liver stem cells from primates such as humans." Applicants respectfully traverse the rejection.

Applicants respectfully note that the claimed invention is a method of obtaining a population of cells enriched in human liver cells comprising, in part, "adjusting the density of the medium in which the cells are suspended whereby *at least two bands of cells separated by a density barrier* are obtained upon centrifugation." (Emphasis added.) A Percoll density gradient, as taught or known at the time of invention (also referred to herein as the "standard" Percoll method), did not yield, upon centrifugation, *at least two bands of cells separated by a density barrier*. In fact, a Percoll density gradient did not produce any bands of cells, let alone two bands that are separated by a density barrier upon centrifugation. A Percoll density gradient as taught in the art, including the copending application, separates cells in three fractions: a pellet at the bottom of the density gradient, afloat atop the density gradient, and interspersed *thoughout* the density gradient (*i.e.*, *not* in a discrete band). Hence, because the '359 application cannot generate at least two bands of cells separated by a density barrier, the reference does not teach each and every limitation of the claimed invention and cannot be held to anticipate the instant invention. Withdrawal of the rejection is therefore respectfully requested.

Claims 1-2 and 6 are newly rejected under 35 U.S.C. § 102(b) as being anticipated by EP682106 to Tateno *et al.* ("Tateno"). The Office alleges that the instant method claims do not exclude other methods of isolating hepatic cells such as a "standard percoll-based isolation

method." In this respect, the Examiner alleges that Tateno teaches the isolation of adult hepatic cells from the "light fraction" upon Percoll centrifugation. Applicants respectfully traverse this rejection as follows.

While the present claims do not *a priori* exclude all methods of isolation using a density gradient comprising Percoll *per se*, the claims necessarily do exclude the *standard* Percoll-based isolation method. The exclusion is not based on the composition of the density gradient *per se*, but on the premise that the standard Percoll-based isolation method (as defined in the art, the cited references, and presumably, the Examiner) does not have a density such that "at least two bands of cells separated by a density barrier are obtained upon centrifugation." As described above, it is well appreciated in the art that the standard Percoll-based isolation method does not result in any bands, let alone two discrete bands as claimed. Unsurprisingly, therefore, Tateno is completely silent on the formation of any bands at all. Rather, one of ordinary skill in the art would appreciate that the "light fraction" referred to in Tateno is the population of cells that float atop the density gradient upon centrifugation and the "heavy fraction" is presumably the pellet. The presently claimed invention, in marked contrast, requires the generation and isolation of cells from the lower density band of two *bands* of cells separated by a density barrier.

Aside from the steps of the claimed method, the standard Percoll method *excludes* the very cells that the claimed invention seeks to isolate (*e.g.*, viable human liver cells, including hepatic stem/progenitor cells). According to the standard Percoll method, cells from the pellet, which is now believed to comprise hepatic stem/progenitor cells, is discarded. Hence, the standard Percoll method fails to (1) meet at least step (c) of the claimed invention and (2) isolate the end-product of the method, namely, a population of cells enriched in viable human liver cells, including hepatic stem/progenitor cells.

In sum, as with the '359 application, Tateno fails to teach each and every limitation of the claimed invention, in particular, the isolation of hepatic progenitor cells from the lower density band of two *bands* of cells separated by a density barrier upon centrifugation. Hence, neither

reference can be held to anticipate the instant invention. Withdrawal of the rejections is therefore respectfully requested.

Claim rejections under 35 U.S.C. § 103

Claims 1-10, 12-17 and 26 are rejected under 35 U.S.C. 103(a) as being unpatentable over Tateno; Singh *et al.*, Acta Physiol Scand 117(4):497-505, April 1983 ("Singh"); and USP No. 5785964 to Naughton *et al.* ("Naughton). The reasons for the rejection may be found on pages 8-11 of the outstanding office action. Applicants respectfully traverse this rejection.

In order to establish a *prima facie* case for obviousness, the Office must establish, inter alia, that each and every element of the claimed invention can be found in the prior art. As emphasized throughout this submission, the claimed invention recites a step of "adjusting the density of the medium in which the cells are suspended whereby at least two bands of cells separated by a density barrier are obtained upon centrifugation, at least one band of the at least two bands being of a lower density than another band of the at least two bands; and collecting the at least one band of lower density." Each of the cited references, however, teaches the use of a standard Percoll gradient, which gradient (as explained above) is not adjusted such that *at least two bands* of cells separated by a density barrier are obtained upon centrifugation. In fact, *no* bands are obtained by the standard Percoll method. Hence, the cited references, alone or in combination, do not arrive at the claimed invention. What is more, because the standard Percoll method can not arrive at the claimed invention (at least with respect to step (c) of claim 1), one or ordinary skill in the art simply could not have had a reasonable expectation of success in using the standard Percoll method to arrive at the claimed invention.

Claims 1-6, 8, 11-17, 22-28 and 88-100 are rejected under the subject statute as being unpatentable over Tateno; Brill *et al.*, Proc Soc Exp Biol Med. 1993;204(3): 261-9 ("Brill"); Cassiman *et al.* Am J Pathol. 1999;155(6): 1831-9 ("Cassiman"); and Graham, Scientific World J 2:1347-50, May 2002 ("Graham"). The reasons for the rejection may be found on pages 11-15 of the outstanding office action. Applicants respectfully traverse this rejection.

Tateno and Brill teach the use of a standard Percoll gradient, which gradient, for the reasons mentioned hereinabove, does not have the advantages of the present invention. In fact, Applicants submit respectfully that Tateno and Brill teach away from the present invention insofar as the standard Percoll method excludes hepatic stem/progenitor cells from the isolate.

Cassiman and Graham recite the use of iodixanol gradients. However, both of these references do not use (and therefore disclose) iodixanol gradients for the isolation of hepatic progenitor cells. As noted by the Examiner, "Cassiman et al. differ[] from the claimed invention by not disclosing isolation of hepatic cell includes [sic] progenitor cells." Applicants also agree with the Examiner that "Graham et al do not teach the [claimed?] method steps to isolate cells." What is more, neither reference teaches "centrifuging ... to obtain at least one band enriched for viable cells" as in claim 94 or at least two bands as in claim 1. In fact, Graham teachs the collection of cells "at the interface between the GBSS [media] and the 11.5% iodixanol (see Fig. 1)." Page 1349, step no. 5 under "stellate cells." Graham also suggests that Cassiman's cells are collected in a similar manner. *See* item no. 1 under "notes," page 1349. Hence, these references alone or in combination cannot properly sustain a *prima facie* case for obviousness as none of the references, alone or in combination, teaches each of the limitations of the claimed invention.

Claims 1-17, 22-28, 88-100 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Tateno, Brill, Cassiman, Graham and Naughton. The reasons for the rejection may be found on pages 15-17 of the outstanding office action. Applicants respectfully traverse this rejection.

Applicants respectfully maintain that none of these five references, alone or in combination, arrives at the presently claimed invention. Specifically, the combination of references, even if held to be proper, fails to teach the isolation of an enriched population of viable human liver cells comprising functional hepatocytes and hepatic stem/progenitor cells by collecting cells from a discrete band formed upon centrifugation. Accordingly, the *prima facie* case for obvious is improperly substantiated.

Taken together and for at least these reasons, Applicants respectfully submit that a *prima facie* case for obviousness has not been established and respectfully request the withdrawal of same rejections.

Double patenting

Claims 1-28 stand provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1, 3, 4, 6-9, 12-2 1, 23-34 of the '359 application. Though the conflicting claims are not identical, they are nonetheless alleged to be patentably indistinct from each other because "the use of percoll gradients for separation of cell populations from the liver [in the two disclosures]...would render the instant set of claims obvious over the other." Applicants respectfully traverse this rejection.

Traversal is based on the grounds that nothing in the art prior to the instant application renders obvious a process of obtaining cells enriched in human liver cells, including hepatic stem and progenitor cells, by density centrifugation resulting in "two bands of cells separated by a density barrier" let alone "collecting the at least one band of lower density." As reiterated above, the present claims necessarily exclude the "standard" Percoll-based methodology of isolating cells insofar as two bands separated by a density barrier is not generated. For this reason and the others expounded above, the present claims are novel and non-obvious (i.e., patentably distinct) in view of the the '359 application.

Applicants believe that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested. The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check or

credit card payment form being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicants hereby petition for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

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By

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